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AN EVALUATION OF THE MEMBRANE CONSTANTS AND THE POTASSIUM CONDUCTANCE IN METABOLICALLY EXHAUSTED MUSCLE FIBRES

BY R. FINK AND H. C. LÜTTGAU
From the Department of Cell Physiology, Ruhr-University, D-4630 Bochum, Germany

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SUMMARY

1. The membrane characteristics of metabolically poisoned and mechanically exhausted frog skeletal muscle fibres were investigated with intracellular micro-electrodes.

2. When cyanide plus iodoacetate were applied as metabolic poisons twitch tension declined towards zero after 150–300 stimuli (0·3 Hz; temperature = 0° C). At the beginning of stimulation the mean resting potential fell from −75 to −69 mV; it rose subsequently to −83 mV. The membrane resistance decreased during this stimulation period along a sigmoid time course to 4–6% of the original value.

3. In completely exhausted fibres the following membrane constants were estimated (23° C): length constant, 0·31 mm; input resistance, 31 kΩ; membrane resistance, 58 Ω.cm². These values were calculated under the assumption of a constant internal resistivity of 170 Ω.cm. The Q_{10} values of these constants were similar to those in normal fibres. A few experiments revealed that the membrane capacity remained roughly constant under these conditions.

4. The current–voltage relation of exhausted fibres was approximately linear in the range between −60 and −100 mV. At less negative potentials the conductance increased slightly while at more negative potentials it decreased. The latter, in particular, became more evident when the input current was converted into membrane current density by applying Cole's theorem.

5. TEA^+ and Rb^+ in the external solution increased the membrane resistance of exhausted fibres by more than one order of magnitude. The major part of the membrane conductance induced by exhaustion, however, could not be blocked by these ions or Zn^{2+}.

6. Chloride-free test solutions were used to measure the relative contributions of potassium and chloride ions to the membrane conductance.

The relation $G_K : G_{Cl}$ changed from 2 : 3 in normal fibres to 5 : 1 in exhausted ones. In absolute terms G_K rose from *ca.* 130 to 14,300 μmho/cm^2 and G_{Cl} from *ca.* 200 to 2900 μmho/cm^2. The discrimination between K^+ and Na^+ by the resting membrane in exhausted fibres was probably equal to or even higher than that under normal conditions.

7. In normal fibres the input resistance decreased by up to 20% after the external application of 1–2 mM caffeine, which is known to release calcium ions from internal stores. The elevation in internal Ca^{2+} by direct injection caused a small and, as a rule, irreversible decrease in input resistance which was probably partly due to local damage to the surface membrane.

8. It is concluded that in metabolically exhausted muscle fibres the surface and tubular membranes are still intact and that the observed decrease in membrane resistance is mainly due to an increase in potassium conductance. In addition, the results indicate that the gating mechanism of the potassium channels (presumably those with the characteristics of the slow component) is affected when energy reserves diminish.

INTRODUCTION

Skeletal muscle fibres whose energy reserves have been drastically reduced during repetitive stimulation have an extremely low membrane resistance (Grabowski, Lobsiger & Lüttgau, 1972). In these fibres the resting potential is normal or sometimes more negative than normal. Action potentials may still be elicited but they have a reduced overshoot and are not followed by after-potentials. From these and further results it has been deduced that these effects are mainly due to a large increase in potassium conductance (Fink & Lüttgau, 1973; Oetliker, 1973). This has been confirmed in principle by the quantitative experiments described here. In addition it has been possible to characterize the conductance change more specifically. The results are discussed in connexion both with recent electrophysiological experiments which reveal different kinds of potassium channel (cf. Adrian, Chandler & Hodgkin, 1970) and with the hypothesis concerning the role of internal Ca as an activator of potassium conductance (cf. Meech, 1974).

A preliminary account of some of the experiments described here was given at a joint meeting of the Austrian and German Physiological Societies at Vienna (Fink, 1975).

METHODS

Preparation and experimental chamber. All experiments described here were performed with sartorius muscles from the frog (*Rana temporaria*). The frogs used were kept in a dark room at 4° C for periods up to several weeks, without feeding. The

muscles were dissected and either transferred immediately to the experimental chamber or stored in a refrigerator for 2–5 hr at 3° C.

In the experimental chamber the muscle was stretched to about 130 % of its slack length and connected to rigid hooks which allowed only isometric contraction of the muscle. Movement artifacts were further reduced in some experiments by applying a method first described by Stefani & Schmidt (1972): the muscles were wrapped around a Perspex rod (diameter *ca.* 3 mm) and stretched to about 150 % of their slack length. The bathing solution (chamber volume about 7 ml.) was exchanged within 100 sec by flushing the chamber with 70 ml. new solution.

Electrical measurements. Membrane potentials were recorded in the usual way with internal glass micro-electrodes and membrane constants were obtained with two internal electrodes by making use of methods ('low frequency cable analysis') described by Fatt & Katz (1951) and Eisenberg & Gage (1969). By applying Laplace's equation Hodgkin & Nakajima (1972*b*) showed that the one-dimensional cable equations can be used as a good approximation even for small space constants, e.g. during the foot of the action potential where the length constant of the fibre, λ_f, is only equivalent to five times the fibre radius. Since the space constant of exhausted fibres, λ_e, lies in the same range as λ_f, the one-dimensional theory was employed.

In some mainly qualitative experiments the potential decay between the point of current injection and the nearest point at which the membrane potential was recorded (*ca.* 30 μm) was not taken into account since only relative values for the input resistance of fibre, R_o, were required. This 'relative' input resistance, R'_o, is defined as

$$R'_o = R\,(x_1) = R_o \cdot e^{-x_1/\lambda}, \tag{1}$$

where the distance along the fibre from the current electrode, $x_1 \doteqdot 30$ μm; R_o is the input resistance at $x_o = 0$. R_o should be underestimated by no more than 10 % in our experiments ($x_1/\lambda < 0{\cdot}1$).

Because of the advantage of the simple recording technique the relation

$$\frac{R'_m\,(1)}{R'_m\,(2)} \doteqdot \left(\frac{R'_o\,(1)^2}{R'_o\,(2)}\right), \tag{2}$$

with $R'_m \doteqdot R_m\,(x_1)$ was used to give a rough comparison of changes of relative input and membrane resistances, R'_m, from a state (1) to a state (2).

For more quantitative measurements the values of the exponential function $R(x) = V_m\,(x)/I_o$ were recorded for several electrode separations, where V_m was the change in potential difference across the surface membrane and I_o the current flowing through the electrode. The linear relation given by the logarithms of $R(x_1)$, $R(x_2)$, . . . was extrapolated to R_o. The length constant, λ, was calculated from the slope of the line and the membrane resistance referred to the surface membrane, R_m, could be determined as

$$R_m = \lambda\sqrt{(\lambda \cdot R_o \cdot 8 \cdot \pi \cdot R_i)}, \tag{3}$$

assuming a constant internal resistivity, R_i (cf. Results, p. 223; Hodgkin & Nakajima, 1972*a*). The 'electrical' fibre diameter, D, for a cylindrical fibre was obtained from

$$D = \sqrt{\frac{(2 \cdot \lambda \cdot R_i)}{(R_o \cdot \pi)}}. \tag{4}$$

Since the sartorius muscle fibre may be assumed to approximate an infinite cable the membrane current density in absolute values, I_m (μA/cm^2), was derived from the steady-state current–voltage relations using Cole's theorem (Cole, 1961)

$$I_m = \frac{R_i}{\pi^2 D^3} \cdot I_o \cdot \frac{dI_o}{dV_m}, \tag{5}$$

where D is the 'electrically' determined fibre diameter. dI_o/dV_m was obtained graphically from the slope of the current–voltage curve.

The membrane capacity referred to the surface, C_m, was estimated from the linear relation of the half-time to the distance between the electrodes (membrane time constant, $\tau = 4{\cdot}398\ T_{\frac{1}{2}}^{\circ}$; Gage & Eisenberg, 1969) and from the 84% value of the passive voltage response at $x = 0$ (erf (1) = 0·84; Hodgkin & Rushton, 1946) in a combined determination of the membrane resistance.

Solutions. The composition of the modified Ringer solutions used are summarized in Table 1. Standard (chloride) Ringer solution (soln. *A*) was the same as that used by Adrian (1956) and the chloride-free sulphate solution, which had about the same ionic strength and the same tonicity as solution *A* was taken from Hodgkin & Horowicz (1959). The ionized Ca in this solution probably amounts to 1 mM as suggested by the authors. The metabolic poisons NaCN and iodoacetate were dissolved in stock solutions which contained (in mM) in the cyanide solution: NaCN, 20; NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; and Tris Cl, 3; and in the iodoacetate solution: 40 mM iodoacetic acid neutralized with NaOH. To obtain the desired dilutions, as a rule 2 mM-CN^- and 1 mM iodoacetate in Ringer solution, solution *A* was mixed with the cyanide stock solution and iodoacetate was finally added from the stock solution. Further substances: Digitoxigenin (Boehringer, Mannheim) was used to block the Na-K-activated transport–ATPase and caffeine (Merck, Darmstadt) to induce the release of Ca ions from the sarcoplasmic reticulum.

Internal application of Ca ions. Ca ions were injected into single fibres with the help of Ca-filled micro-electrodes, either iontophoretically or by applying an air pressure of 1·0–4·5 atm. The electrodes with a tip diameter of 1·5–3 μm were filled with 20 mM-$CaCl_2$ plus 100 mM-KCl.

Temperature. Most of the experiments were performed at room temperature (20–24° C) or at a low temperature near 0° C. The temperature was checked via a thermistor. The recording chamber was enclosed by a cooling jacket, which held the temperature constant with a maximal deviation of ± 0·5° C.

Evaluation of the experimental results. A Hewlett Packard calculator 9100A was used for the calculation of the cable constants and the statistical procedures. In most cases the lines were fitted by linear regression. The mean values are given ± standard error of the mean, S.E. of mean, n being the number of experiments.

RESULTS

Resting potential, action potential and input resistance of poisoned muscle fibres during repetitive stimulation (0·3 *Hz*)

The membrane characteristics of muscle fibres change drastically when they are stimulated, in particular when their energy resources have been partially blocked by metabolic poisons such as cyanide (CN^-) and iodoacetate. In the following, the time course of these alterations is shown by describing in detail a representative experiment.

A sartorius muscle was wrapped around a Perspex rod and placed in the experimental chamber as described under Methods. The bathing solution (solution *A*, Table 1) included 2 mM-CN^- and 1 mM iodoacetate and was cooled to 0° C. This combination of poisons was chosen with the intention of blocking both glycolysis and oxidative metabolism. Before starting the experiment the muscle was left in this solution for 1 hr. At a separation of

TABLE 1. (*a*) Composition of the modified standard Ringer solutions (concentrations in mM)

	NaCl	KCl	$CaCl_2$	Na_2HPO_4	NaH_2PO_4	Tris Cl	TEA Cl	RbCl	Zn acetate	Caffeine	Sucrose
A	115	2·5	1·8	2·15	0·85	—	—	—	—	—	—
B	—	2·5	1·8	—	—	3	115	—	—	—	—
C	115	—	1·8	—	—	3	—	2·5	—	—	—
D	115	—	1·8	—	—	3	115	2·5	—	—	—
E	115	2·5	1·8	—	—	3	—	—	2·5	—	—
F	—	2·5	1·8	—	—	3	115	—	2·5	—	—
G	—	2·5	1·8	—	—	3	115	2·5	2·5	—	—
H	115	2·5	1·8	—	—	3	—	—	—	1–2	350
I	115	2·5	1·8	2·15	0·85	—	—	—	—	—	350

A, standard Ringer solution (chloride Ringer solution); *B*, TEA Ringer solution, *C*, rubidium Ringer solution; *D*, TEA–Rb Ringer solution; *E*, Zn acetate Ringer solution; *F*, TEA–Zn acetate Ringer solution; *G*, TEA–Zn acetate–Rb Ringer solution; *H*, hypertonic caffeine Ringer solution; *I*, hypertonic Ringer solution. All solutions were adjusted to a pH of 7·2 with NaOH or HCl.

(*b*) Composition of the chloride-free sulphate solution (concentrations in mM)

K_2SO_4	Na_2SO_4	Sucrose	$CaSO_4$	Na_2HPO_4	NaH_2PO_4
1·25	38·75	113	8	1·08	0·43

30 μm two micro-electrodes, one for injecting current and one for measuring the membrane potential, were inserted into a fibre. This fibre was then stimulated with external electrodes at 0·3 Hz. About 300 msec after each stimulus the membrane input resistance was checked by injecting a constant hyperpolarizing current of 0·12 μA and 700 msec duration into the fibre.

As can be seen in Fig. 1*A* the resting potential at the beginning of the stimulation period was relatively low (-76 mV). This was probably due to a decrease in the internal potassium concentration (as a result of the applied metabolic poisons), the reduction in resting potential usually observed when the temperature is lowered (Hodgkin & Nakajima, 1972*a*) and the leak caused by the electrodes. The mean membrane potential of nine fibres treated in this way was -75 ± 2 mV. In the experiment shown in Fig. 1 the membrane depolarized by about 2 mV during the first fifty stimuli (mean depolarization in five fibres 6 ± 2 mV). Then, however, it started to hyperpolarize and after about 150 stimuli it reached a maximum value of -84 mV (mean value for five fibres -83 ± 1 mV). This hyperpolarization was not affected by the addition of 10^{-6} g/ml. Digitoxigenin which is sufficient to inhibit the activity of the Na–K-ATPase. The low temperature (0° C) and the blockade of energy resources by CN^- and iodoacetate provide additional arguments against the assumption that the hyperpolarization was caused by an electrogenic sodium extrusion (see Adrian & Slayman, 1966).

On technical grounds, an electronic device was used to reduce the action potential at the chart recorder to 20–50% of the true value. Thus, Fig. 1*A* only shows whether or not an action potential was induced. For this reason the action potentials were recorded in parallel on an oscilloscope (Fig. 1*B*). It can be seen from Fig. 1*B* (*a*, *b* and *c*) that during the first 100 stimuli the overshoot declined by only a few millivolts while the duration of the action potential and the amplitude of the early after-potential (transiently) increased (cf. Persson, 1963). This tendency reversed, however, with the beginning of the hyperpolarization and the reduction in input resistance. The action potential became shorter, the overshoot fell more rapidly and the after-potential almost completely disappeared (see also Fig. 4 of Grabowski *et al.* 1972). Finally, it was no longer possible to induce propagated action potentials (Fig. 1*B*, *i*). This happened in most cases when the temperature was low. In experiments conducted at room temperature, however, propagated action potentials could sometimes be induced. According to calculations of Cole (1968) a resting membrane conductance of 8·6 m-mho/cm^2 is the critical conductance for the induction of propagated action potentials in squid giant axons. The membrane conductance of exhausted muscle fibres was probably lower but of the same order of

magnitude. The effect of temperature upon the critical membrane conductance might be explained with a different temperature dependence of the kinetics of the sodium and potassium conductances.

The input resistance R'_o (cf. Methods, p. 217) declined to about one

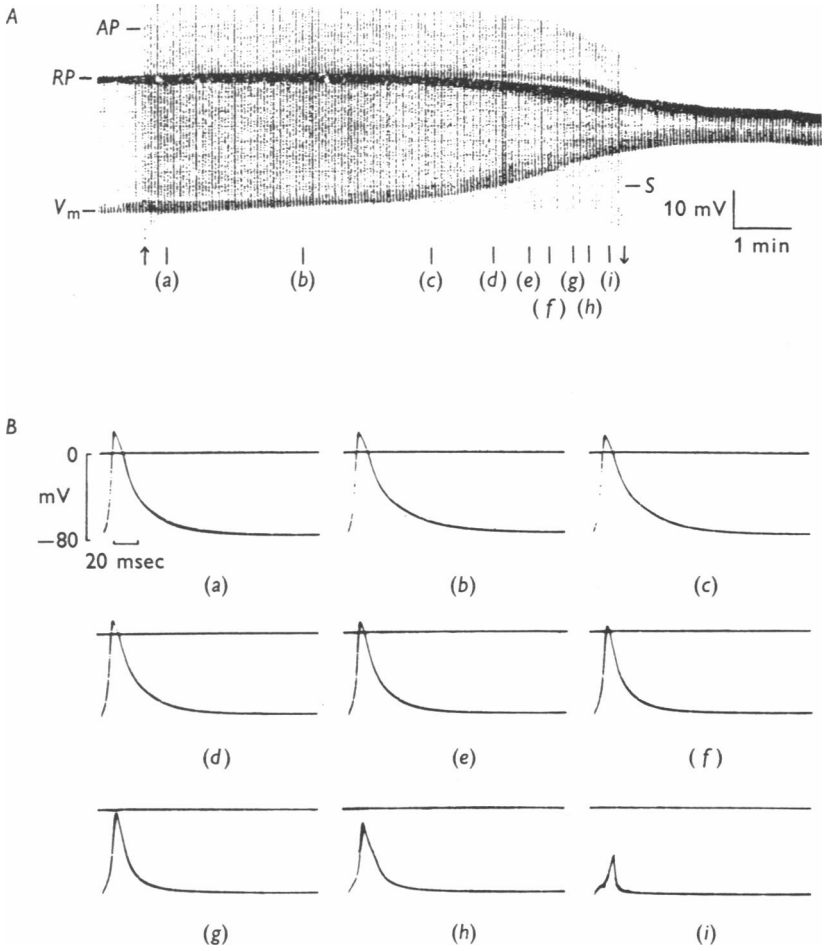

Fig. 1. *A*, resting potential (*RP*) and action potentials (*AP*) of a metabolically poisoned muscle fibre during repetitive stimulation at 0·3 Hz (chart recorder trace). The resting potential started at −76 mV and rose to −84 mV by the end of the trace. Downward deflexions are the potential shifts (V_m) caused by a constant hyperpolarizing current (0·12 μA) of 700 msec duration, 10 mV ≡ 83·4 kΩ. *S* = stimulation artifacts; ↑, ↓, stimulation period. (For further details see text).

B, action potentials from the experiment shown in *A* recorded separately with an oscilloscope using a faster time base. (*a*)–(*i*) Time (see *A*) when the action potential was taken. Temperature, 0° C.

quarter of the original value (Fig. 1*A*). This corresponds to a reduction of the membrane resistance, R'_m (see following section), to values as low as 4–6% of normal. Fig. 2 shows the time course of the decline in resistance of four different fibres. It follows a sigmoid curve to a stable, and for all fibres nearly the same, low value. Variable energy reserves and the geometry of the fibres might be responsible for the differing length of the plateau phase.

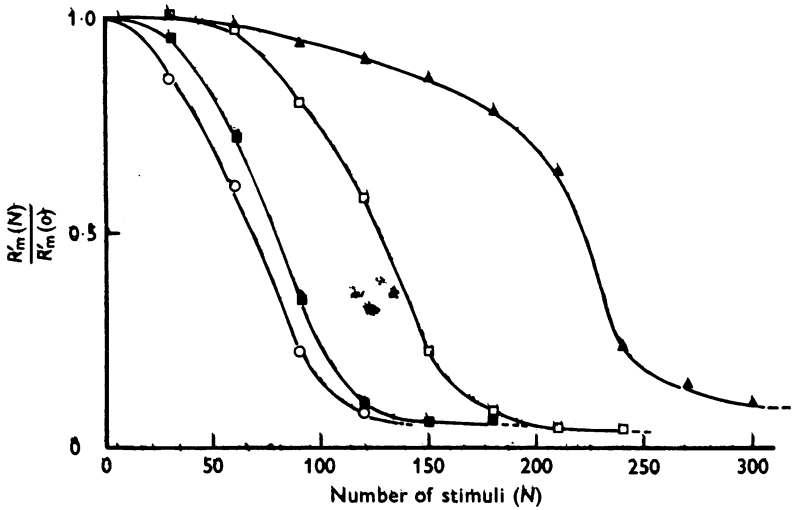

Fig. 2. The dependence of the membrane resistance (in relative units) of four different fibres on the number of stimuli (N). Ringer solution with 2 mM-CN^- and 1 mM iodoacetate. R'_m (o) membrane resistance before stimulation; R'_m (N) membrane resistance after N stimuli; ■, represents the experiment which has been described in detail. Temperature, 0° C.

Analysis of the cable properties of completely exhausted muscle fibres

The experiments described previously only give qualitative features of the changes in the electrical parameters of exhausted muscle fibres. For a more quantitative analysis the space constant of exhausted fibres was measured in addition to the input resistance. The procedure to induce complete exhaustion was started by poisoning the stretched muscle in Ringer solution with 2 mM-CN^- and 1 mM iodoacetate for 1 hr. Then the muscle was stimulated at a frequency of 1 Hz until twitches could no longer be observed under the binocular microscope (forty times magnification). Complete exhaustion (as defined by the procedure above) was reached at 0–1° C after about 300–800 stimuli and at 23° C after about 120–400 stimuli. Finally the exponential shape of the electrotonic potential decay, from which the membrane constants were calculated, was construc-

ted from at least four different separations between the current passing and the potential recording electrodes.

The results are summarized in Table 2, whereby the index *e* marks the parameters of exhausted fibres. The calculation of the fibre diameter, D_e, and the membrane resistance, R_{me}, was based on the assumption that the specific internal resistivity of normal fibres, R_i, remains constant at a value of 170 Ω.cm at 20° C with a Q_{10} of 1·37 (Hodgkin & Nakajima, 1972*a*). This assumption was suggested by optical measurements as described in the following paragraph.

The electrically determined fibre diameters in exhausted muscles, D_e, increased to values of 117 ± 6 μm (0 – 1° C) and 105 ± 7 μm (23° C), respectively, compared to the 85 μm diameter of normal fibres (Hodgkin & Nakajima, 1972*a*). A comparable swelling of the fibres which seems to develop in the exhausted state could be found with measurements of fibre diameters from photographed muscle sections (F. Göbelsmann, unpublished; R. Fink, F. Göbelsmann & H. C. Lüttgau, in preparation). Gonzáles-Serratos, Borrero & Franzini-Armstrong (1974) reported a 1·3-fold increase of the diameter of fatigued frog fibres. This agreement between optical and electrical data supports the assumption that the internal resistivity, R_i, is largely unaltered during exhaustion. If in spite of this argument a decrease of R_i were responsible for an over-estimate of the fibre diameter, the already extremely low membrane resistance should be still smaller which does not seem very likely.

The temperature dependence of the length constant, λ_e, the input resistance, R_{oe}, and the membrane resistance, R_{me}, were calculated from the mean values given in Table 2*A* (0–1° C) and *B* (23° C). The temperature coefficients of these electrical constants of exhausted fibres agree essentially with those of normal fibres analysed by Hodgkin & Nakajima (1972*a*). The temperature coefficient of the input resistance, R_{oe}, was about 1·3. The highest temperature dependence was found for the membrane resistance, R_{me}, with a Q_{10} of 1·50. Only the length constant, λ_e, showed little temperature dependence; the Q_{10} was smaller than 1·1.

The electrical values under exhaustion (cf. Table 2*B*) may be compared with those given by Hodgkin & Nakajima (1972*a*) for normal sartorius fibres (diameter 80 μm; 20° C). The comparison reveals that the length constant of exhausted fibres ($\lambda_e = 0{\cdot}306 \pm 0{\cdot}017$ mm, $n = 10$) is less than 20% of that of normal fibres ($\lambda = 1{\cdot}9$ mm). The input resistance ($R_{oe} = 30{\cdot}9 \pm 3{\cdot}9$ kΩ; $n = 10$) was diminished to less than a tenth of normal ($R_o = 320$ kΩ). During stimulation to exhaustion, R_m underwent the most extreme change, falling from an average of 3000 to 58 ± 4 Ω.cm² ($n = 10$).

Since the time constant of exhausted fibres, τ_e, was markedly reduced,

the membrane capacity, C_{me}, could not directly be estimated from the time dependence of the electrotonic potential due to the more pronounced influence of the capacitive artifact from the recording system. Therefore,

TABLE 2. Electrical constants of exhausted sartorius muscle fibres

(I) Fibre	(II) Resting potential (mV)	(III) λ_e (mm)	(IV) R_{oe} (kΩ)	(V) R_{me} (Ω.cm²)	(VI*) D_e (μm)
		A, temperature 0–1° C			
11	−72	0·435	54·5	187	126
12	−86	0·400	56·0	167	118
13	−79	0·236	88·2	95	72
21	−82	0·359	55·9	142	112
22	−74	0·302	48·2	101	112
23	−73	0·383	56·7	158	116
24	−70	0·384	43·1	138	132
31	−82	0·492	56·9	230	130
32	−68	0·361	45·2	128	126
33	−82	0·341	94·8	171	84
42	−80	0·433	42·4	164	142
43	−87	0·296	31·2	79	136
Mean	−78	0·369	56·1	147	117
± S.E. of mean	2	0·020	5·3	12	6
		B, temperature 23° C			
302	−76	0·257	28·4	44	96
303	−74	0·325	23·5	57	118
304	−77	0·307	50·5	77	78
305	−74	0·246	35·6	46	84
306	−79	0·253	54·9	60	68
311	−95	0·304	21·7	50	120
312	−96	0·271	23·8	43	108
313	−89	0·302	24·4	52	112
314	−84	0·402	19·7	72	144
315	−97	0·390	26·9	80	122
Mean	−84	0·306	30·9	58	105
± S.E. of mean	3	0·017	3·9	4	7

* D_e is the electrically determined fibre diameter (cf. Methods, p. 217).

the membrane resistance was increased by applying a TEA–Rb-solution (cf. p. 229 and Table 1, solution *D*) and by lowering the temperature to 0° C. Under these conditions R_{me} rose in four experiments to *ca.* 900 Ω.cm². The membrane capacity was estimated as $8{\cdot}9 \pm 1{\cdot}1$ μF/cm² ($n = 4$). Thus no significant alteration in the electrical connexion between the membrane of the T-system and the surface membrane seems to occur.

Current–voltage relation after metabolic poisoning and exhaustion

Fig. 3*A* shows the current–voltage relation of a poisoned muscle which had been stimulated to complete exhaustion (■) in comparison to that of a normal muscle (▲). The curves were constructed from the steady-state value of the change in membrane potential at the end of 700 msec current pulses. The current–voltage relation of the exhausted fibre was corrected for the potential decay by the extrapolation of R'_0 to R_0 (cf. Methods, p. 217). The membrane current density was calculated from the curves in Fig. 3*A* and plotted in Fig. 3*B* by applying Cole's theorem. The most striking difference between the normal and the exhausted fibre is a tendency to 'linearization' of the current–voltage curve in the latter case in addition to the large increase in conductance. But it should be pointed out that a non-linearity still exists in exhausted fibres: a more or less pronounced increase of the outward conductance by depolarizing steps to less negative potentials, whereas the inward conductance becomes markedly smaller with large hyperpolarizations. These characteristics are more clearly demonstrated in the Cole plots of the membrane current density (Fig. 3*B*). In normal fibres the decrease in conductance during strong hyperpolarizations (which is partly masked by the strong influence of the chloride conductance; cf. the curves for the normal fibre in Fig. 3) is explained by a diminished potassium conductance (Almers, 1972*a*, *b*; Nakajima, Iwasaki & Obata, 1962) and this explanation might also hold for exhausted fibres (see Discussion).

Increase of the lowered input resistance, R'_{oe}, by TEA^+, Rb^+ and Zn^{2+}

The high membrane conductance of exhausted muscles raises the question of the extent to which it is still possible to block the channels involved. To answer this question, experiments were conducted with tetraethylammonium chloride (TEA Cl), rubidium chloride and zinc acetate (Fig. 4) which have well-known blocking effects on the potassium and chloride systems of skeletal muscle (Adrian, 1964; Stanfield, 1970*a*, *b*). The muscles used in these experiments were stimulated to exhaustion in Ringer solution with 2 mM-CN^- plus 1 mM iodoacetate as described previously.

TEA^+. The relative input resistance, R'_{oe}, of an exhausted muscle fibre was measured with a chart recorder first in standard and then in Ringer solution with 115 mM-TEA (composition see Table 1). The input resistance, R'_{oe}, in this fibre increased to a value 2·3 times that in chloride Ringer solution (Fig. 4*A*). The mean increase of R'_{oe} in five experiments was $121 \pm 6\%$. Thus the membrane resistance, R'_m, should reach nearly five

times the normal value by the addition of TEA ions (see eqn. (2)). In related voltage-clamp experiments on normal muscle fibres Stanfield (1970*b*) found an increase in the resting membrane resistance, R_m, from 3530 to 5580 $\Omega.cm^2$ (temperature, 20–23° C). The resting membrane potential of the exhausted fibres was lowered by 5–12 mV (temperature,

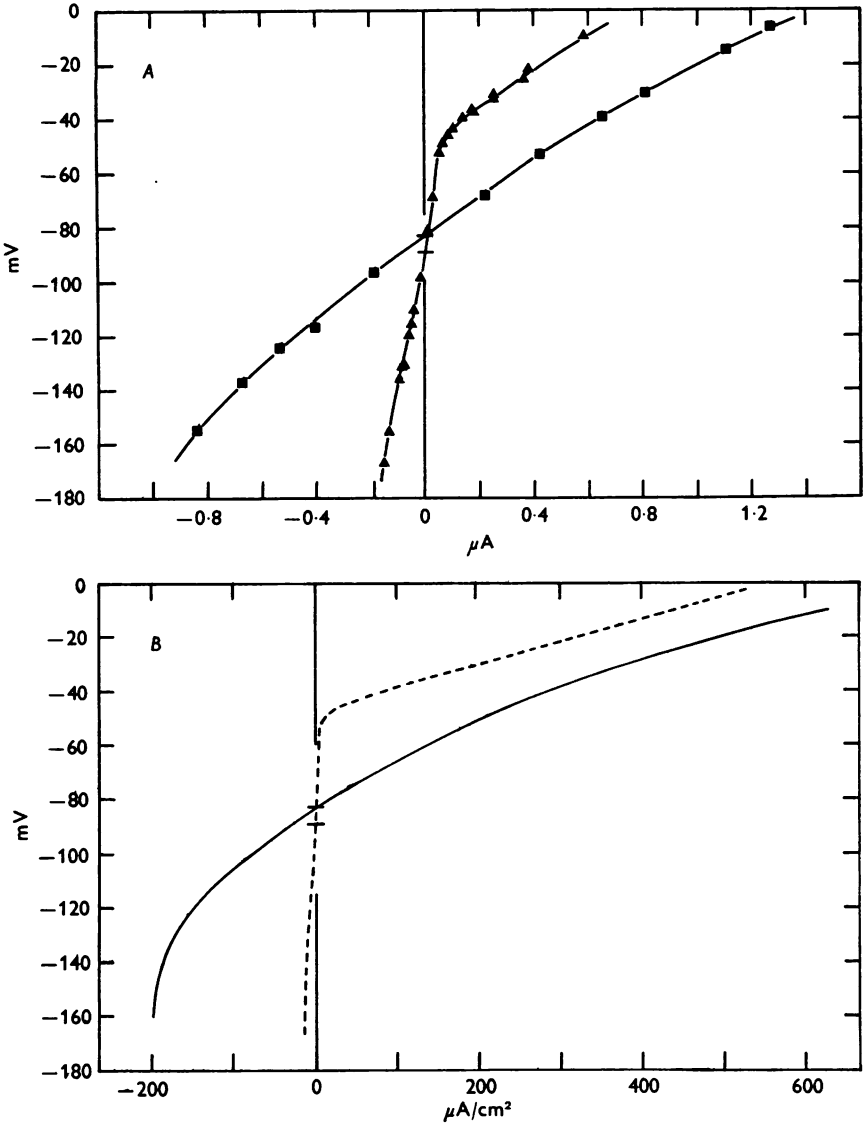

Fig. 3. For legend see facing page.

20° C). This depolarization indicates in addition the decrease of the potassium conductance by TEA ions.

In Fig. 5*A* the current–voltage relation of a poisoned and exhausted muscle fibre (■) is compared to that of the same fibre after the addition of TEA ions (▲). The curve in TEA^+ is even more linearized than that before the reduction of membrane conductance by the blocking agent. This effect is strengthened for inward currents. The results agree qualitatively so far with those obtained in normal, non-exhausted fibres as in both cases inward and outward currents are reduced by TEA ions. In normal and in exhausted fibres the action of TEA^+ is reversible.

Rb^+. Rb ions do not pass the inward rectifying potassium system of skeletal muscle appreciably so that the inward current is reduced (Adrian, 1964). In order to estimate the influence of the inward rectification, the input resistance of exhausted fibres, R'_{oe}, was measured with hyperpolarizing pulses in Rb–Ringer solution in which 2·5 mM-KCl was replaced by RbCl (cf. Table 1, solution *C*). After the exchange of standard Ringer solution by that with Rb ions (Fig. 4*B*) R'_{oe} reached a 1·8-fold higher value (temperature, 22° C). In four experiments the average increase of R'_{oe} amounted to $78 \pm 8\%$ corresponding to a 3·2-fold larger value for R'_{me}.

The current–voltage relation (Fig. 5*B*) in Rb–Ringer solution (●) is characteristically different from that in standard Ringer solution (■). When the exhausted muscle was incubated in the Rb solution for about 10 min the conductance for outward current was only slightly diminished whereas it was substantially reduced for inward current. The current–voltage relation inflects as is to be expected for an inhibition of an inward rectifying component of conductance.

Zn^{2+}. If it is assumed that Rb^+ and TEA^+ act specifically on the potassium

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Fig. 3. Current–voltage relation of a muscle fibre after metabolic poisoning and exhaustion. Temperature, 0° C.

A, steady-state values of the potential change, V_m, at the end of 700 msec current pulses of a muscle which had been stimulated at 1/sec to exhaustion in Ringer solution with 2 mM-CN^- plus 1 mM iodoacetate (■, $D_e = 106$ μm, $\lambda_e = 0{\cdot}434$ mm) and those of a normal fibre (▲, $D = 94$ μm, $\lambda = 2{\cdot}180$ mm, $R_i = 400$ Ω.cm) in a hypertonic Ringer solution with 10^{-7} g/ml. tetrodotoxin. The resting potentials (——) were −83 mV in the exhausted and −89 mV in the normal fibre. Ordinate, membrane potential (mV); abscissa, total current (μA).

B, membrane current density (I_m) with respect to the membrane potential. I_m has been derived from an analysis of the total current curves of *A* by applying Cole's theorem. Continuous line, exhausted fibre; dashed line, normal fibre. Ordinate, membrane potential (mV); abscissa, membrane current density (μA/cm²).

system of exhausted muscle one should also examine whether the chloride conductance can be separately inhibited. In normal muscle lowering the pH from 7·2 to 5·6 in the external solution causes a large fall in the chloride conductance (Hutter & Warner, 1967*a*, *b*). In preliminary experiments on exhausted muscles it was found that the high membrane conductance was not significantly altered when the pH was lowered to the same extent

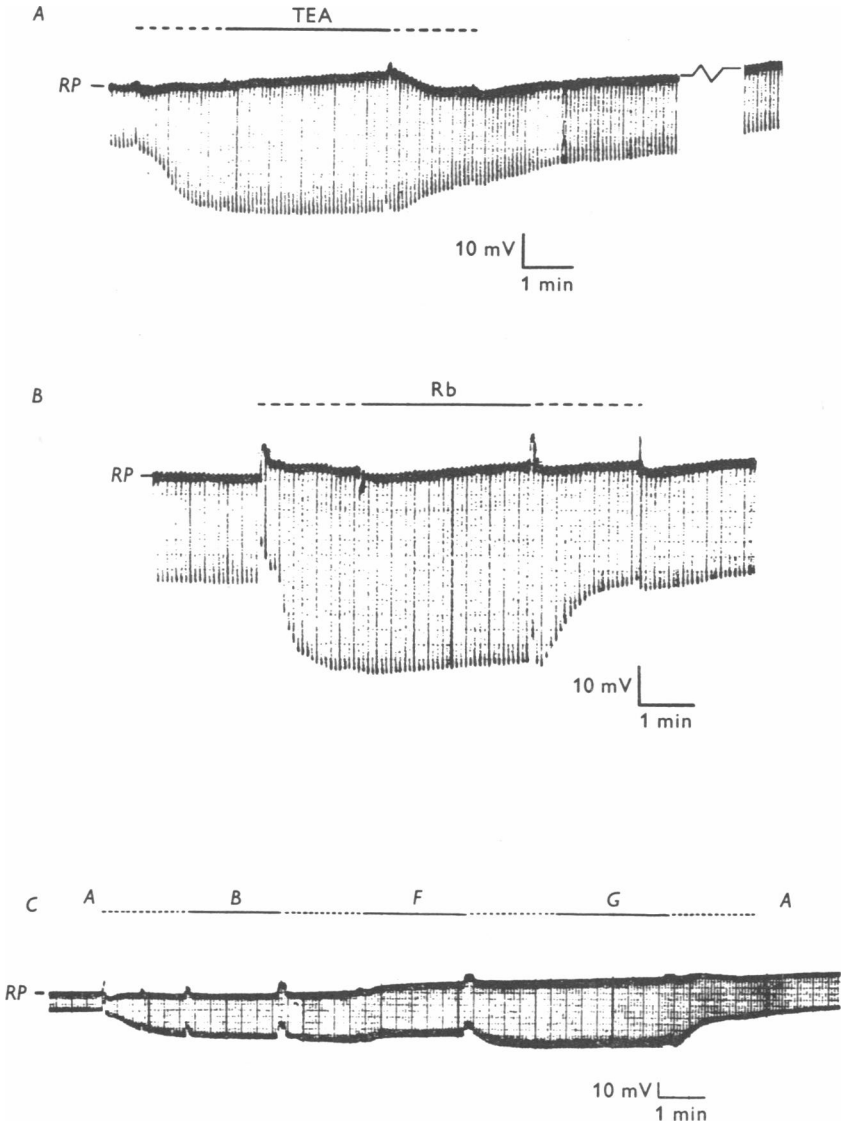

Fig. 4. For legend see facing page.

(Fink & Lüttgau, 1973). This result might be due to the negligible influence of the chloride conductance or to a loss of its pH-sensitivity in exhausted fibres. In order to distinguish between these possibilities the action of Zn ions on exhausted muscles was tested since Stanfield (1970 *b*) attributed the reduction of the normal resting membrane conductance in a 2·5 mM Zn acetate Ringer solution mainly to a fall in chloride conductance.

The membrane resistance during exhaustion, R'_{me}, increased by only 20–45% in 2·5 mM-Zn^{2+} Ringer solution (cf. Table 1, solution *E*; temperature, 23° C) while Stanfield (1970*b*) described that in a similar solution the membrane resistance of normal fibres reached about twice the value in Ringer solution. The weaker inhibition of the high resting membrane conductance in exhaustion suggests that the contribution of chloride ions to membrane conductance is relatively small. Additional arguments, supporting this suggestion, are given by experiments in which the chloride ions were replaced externally by sulphate ions (see p. 234).

Combined effects of TEA^+, Rb^+ and Zn^{2+}. In another series of experiments the low input resistance was increased by using combinations of TEA, Rb and Zn ions in the bathing solution. An example is shown in Fig. 4*C*. First the resistance R'_{oe} increased by a factor of 2·3 in 115 mM-TEA^+ Ringer solution. Then the bathing solution was replaced by one with 2·5 mM-Zn^{2+} plus 115 mM-TEA^+ (cf. Table 1, solution *E*) and the input resistance reached 2·8 times that at the beginning of the experiment in the chloride Ringer solution. The subsequent addition of Rb ions (cf. Table 1, solution *F*) resulted in a further increase in resistance; R'_{oe} reached 3·7 times the initial value in the exhausted fibre. The membrane resistance, R'_{me}, should therefore have increased by a factor of 13·7. If the 'channels' in the exhausted fibres had a specificity similar to that in normal muscle the

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Fig. 4. Effects of TEA, Rb and Zn ions on the input resistance of poisoned and exhausted muscle fibres. The input resistances (R'_{oe}) were measured with hyperpolarizing current steps (duration 700 msec, frequency 0·2 Hz in *A* and *B*, 0·5 Hz in C). Continuous line, incubation time in the modified solution; dashed line, period of exchange from one solution to another. *RP* = initial resting potential.

A, increase of R'_{oe} by 115 mM-TEA^+ $RP = -93$ mV, $I_o = 0{\cdot}57$ μA. The break in the trace indicates a time gap of 3 min in which the standard Ringer solution was replaced again. 10 mV ≡ 17·6 kΩ. Temperature, 20° C.

B, increase of R'_{oe} by 2·5 mM-Rb^+. $RP - 83$ mV. $I_o = 0{\cdot}51$ μA. 10 mV ≡ 19·5 kΩ. Temperature, 22° C.

C, increase of R'_{oe} by applying combinations of TEA^+, Rb^+ and Zn^{2+}. $RP = -93$ mV, $I_o = 0{\cdot}38$ μA. Solutions: *A*, standard Ringer solution; *B*, 115 mM-TEA^+; *F*, 115 mM-TEA^+ + 2·5 mM-Zn^{2+}; *G*, 115 mM-TEA^+ + 2·5 mM-Zn^{2+} + 2·5 mM-Rb^+; composition as Table 1. 10 mV ≡ 26·4 kΩ. Temperature, 21° C.

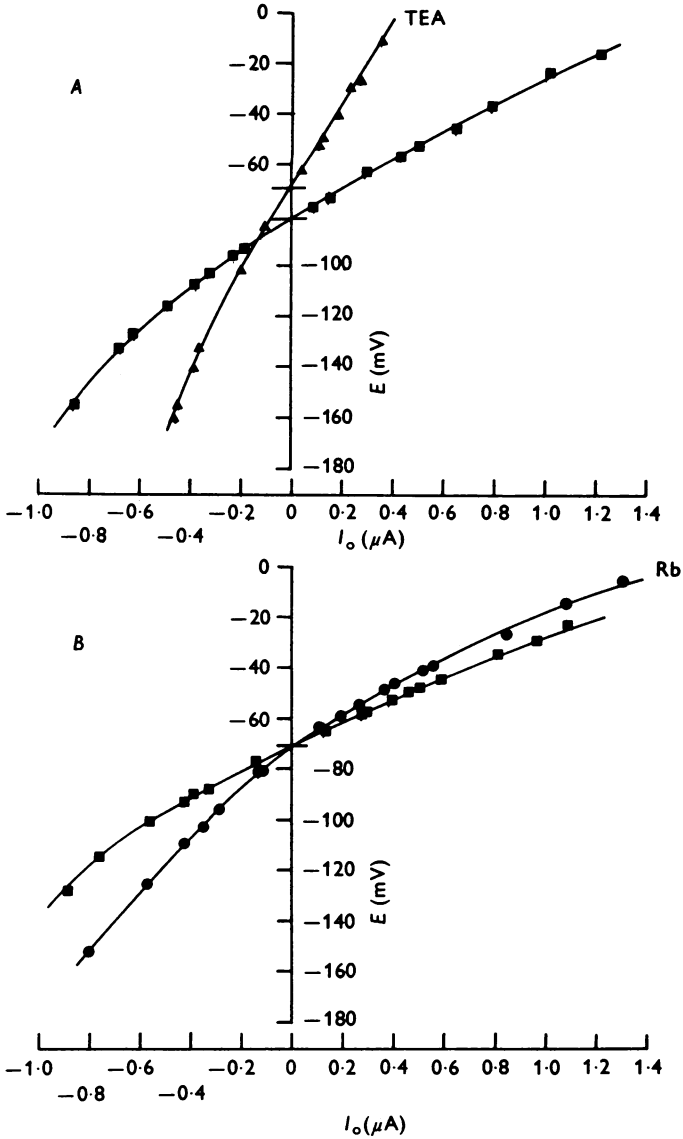

Fig. 5. Current–voltage relations after the reduction of the high membrane conductance of poisoned and exhausted muscle fibres by TEA and Rb ions. The change in the membrane potential, V_m, was recorded at the end of current pulses lasting for 700 msec. Ordinate, absolute membrane potential in mV; abscissa, total current, I_o, in μA. *A*, inhibition in a solution with 115 mM-TEA^+. The curve in standard Ringer solution (■) started from a resting potential of −81 mV and that for Ringer solution with 115 mM-TEA^+ from −69 mV (▲; Table 1, solution *B*). *B*, inhibition in a solution with 2·5 mM-Rb^+ (solution *C*). The fibre had a resting potential of −71 mV in both normal (■) and Rb-Ringer solution (●). Temperature, 0° C.

strong, partly additive inhibitory effects of TEA and Rb ions would indicate that the potassium conductance is mainly responsible for the high membrane conductance of exhausted muscles. It is interesting that even in the presence of these blockers the membrane resistance, R'_{me}, is still significantly lower than that of a normal fibre in Ringer solution. This means that a large part of the high membrane conductance is not affected by TEA, Rb or Zn ions (cf. Discussion, p. 236).

Alterations in membrane resistance of normal muscle fibres after increasing the internal free calcium concentration

Recently Meech (1972, 1974) and Krnjević & Lisiewicz (1972) have described an increase in potassium conductance after the injection of calcium ions into nerve cells. Meech (1974), for example, found that the injection of a calcium–EGTA (ethane dioxy *bis* (ethylamine) tetra-acetic acid) buffer with 9×10^{-7} M free calcium into *Helix aspersa* neurones reduced the membrane resistance by 25%. When $CaCl_2$ was used it was necessary to increase the total intracellular calcium concentration by about 1 mM to produce a similar change in resistance. It can be assumed that in energetically exhausted muscle fibres the internal free calcium concentration rises which on the basis of the cited publications might be regarded as the cause of the observed increase in potassium conductance. This hypothesis, discussed by Fink & Lüttgau (1973), was tested in two series of experiments.

In the first set of experiments calcium was injected into normal muscle fibres either iontophoretically or by air pressure. The electrodes contained a solution with 20 mM-$CaCl_2$ (see Methods). The injection caused a small reduction in R'_m, the membrane resistance, of not more than 10% (0° C). Since the effect was not always fully reversible and probably partly due to local damages of the surface membrane near the electrodes, these experiments were abandoned.

In the second series caffeine, which is thought to release calcium from the sarcoplasmic reticulum (cf. Weber & Herz, 1968), was added to the external solution. In most fibres the concentration used (1–2 mM) was below the threshold for the initiation of a spontaneous contracture (cf. Lüttgau & Oetliker, 1968). The temperature was kept low (0–4° C) to slow down the re-accumulation of calcium by the reticulum. When caffeine was added a slight depolarization was measured; in a few fibres the potential shift reached 10 mV. The input resistance, R'_0, decreased reversibly by up to 20% and on some occasions by 30%. The results are comparable with those found by Axelsson & Thesleff (1958), who described a similar reduction of the input resistance by 3–4 mM caffeine in both normal Ringer solution and a chloride-free 95 mM-K_2SO_4 solution. They attributed this effect to membrane damage produced by the micro-electrodes.

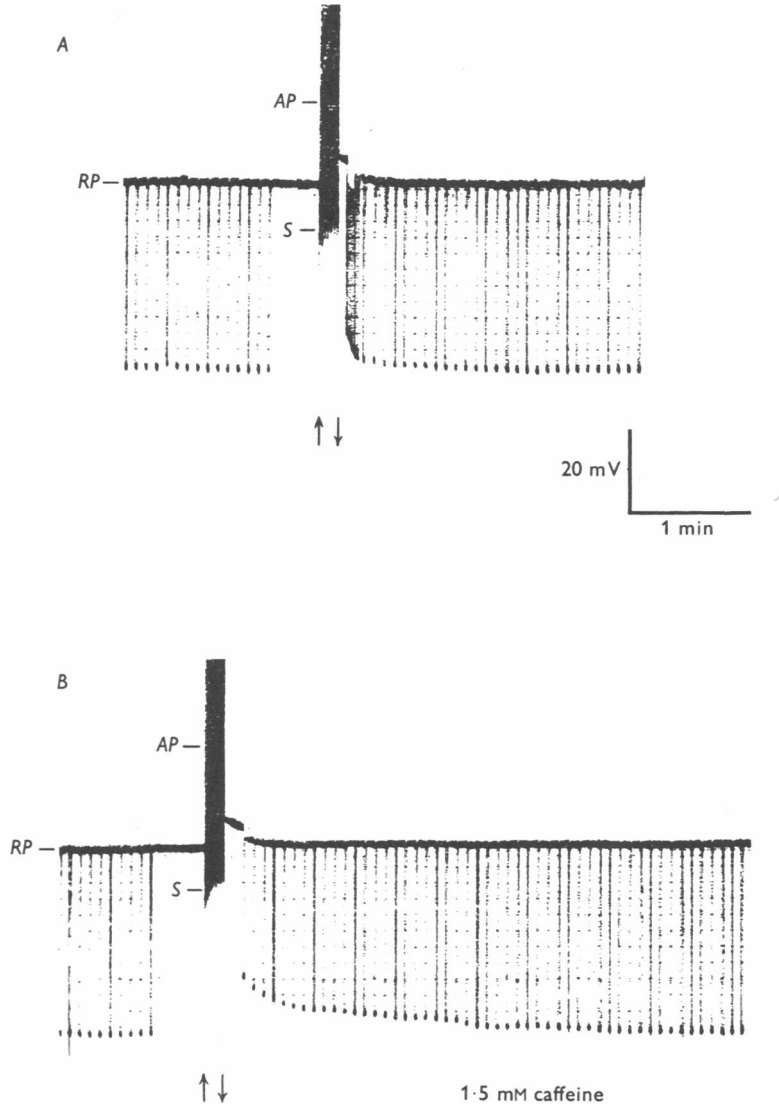

Fig. 6. *A*, input resistance (R'_o) before and after repetitive stimulation; *B*, same fibre with 1·5 mM caffeine in the external solution. The experiment was performed in hypertonic solutions (solution *H*, *I*; Table 1) to reduce movement artifacts. Resting potential, −72 mV; ↑ ↓, stimulation period, *ca.* 10 sec at 5 Hz; hyperpolarizing current pulses (0·066 μA; 20 mV ≡ 303 kΩ) at 0·2 Hz (in *A* first 5 sec after stimulation at 5 Hz); *S* = stimulation artifact; *AP*, action potential (reduced, see Fig. 1*A*); temperature, 4° C.

In Fig. 6*B* a muscle fibre was subsequently stimulated for 10 sec at 5/sec. Shortly after the stimulation the input resistance was 27 % smaller than that measured before the stimulation period (696 → 510 kΩ). It recovered in two phases, a fast component with a time constant of about 5 sec and a slow component with one of 70 sec. In three additional experiments similar results were obtained. When the experiment was performed without caffeine a smaller decrease in membrane resistance was found and the original resistance was restored within 50 sec (Fig. 6*A*).

The results described here are consistent with the hypothesis outlined at the beginning of the section. However, it has to be noted that the experimental situation at the end of a tetanus is obscured by the accumulation of potassium in the extracellular space. Local damage to the membrane must also be considered so that only the qualitative statement is justified, that under these experimental conditions internal calcium probably causes a relatively small increase in potassium conductance.

The relative contribution of potassium and chloride systems to the total conductance in exhausted muscle fibres

Potassium and chloride are the major ions carrying current through the resting membrane of normal muscle fibres. The total conductance, G_m, can thus be represented as the sum of the conductances for these two ions:

$$G_m = \frac{1}{R_m} = G_K + G_{Cl}.$$

Hodgkin & Nakajima (1972*a*) estimated the electrical constants of isolated muscle fibres consecutively in normal and sulphate Ringer solution. Under the assumption of an unaltered sarcoplasmic conductivity they calculated a decrease in normal conductance to 41%, an increase in capacity by 12 % and a reduction in fibre diameter to 92%. If sulphate is regarded as an impermeable ion the experiments indicate that potassium contributes two fifths and chloride three fifths to the total conductance, in agreement with former results (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960).

The replacement of chloride by sulphate is accompanied by a sudden depolarizing shift towards the new chloride equilibrium potential. Subsequently KCl and water leave the fibre which brings the chloride equilibrium potential and the membrane potential back towards the original value.

The relative contribution of chloride to the total conductance is smaller in exhausted fibres. In the experiment shown in Fig. 7 membrane potential and input resistance were continually measured before, during and after the temporary replacement of Ringer solution by a chloride-free sulphate solution (Table 1). The input resistance increased in the absence of

chloride by a factor of 1·08. The mean value from five experiments of this kind was $1{\cdot}12 \pm 0{\cdot}03$. Assuming a reduction in fibre diameter similar to that observed by Hodgkin & Nakajima (1972*a*) in normal fibres under similar conditions (see above) the membrane conductance in the sulphate solution, G_{Ke}, amounts to $83 \pm 4\%$ ($n = 5$) of the mean value in normal Ringer solution. Under the assumption that the membrane in exhausted fibres is still impermeable to sulphate ions the result confirms former conclusions (cf. Fink & Lüttgau, 1973) that the contribution of chloride to

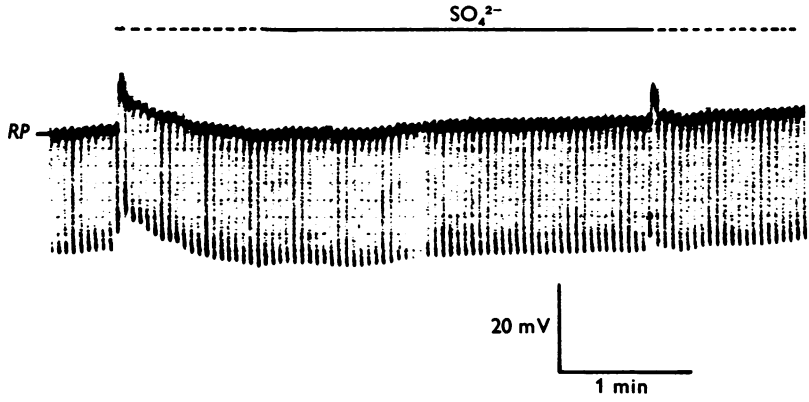

Fig. 7. Increase of the input resistance, R'_{oe}, in chloride-free sulphate solution. The initial resting potential, RP, was -78 mV. R'_{oe} was measured via hyperpolarizing current pulses (frequency *ca.* 0·3 Hz, $I_o = 0{\cdot}6$ μA, duration 700 msec). Continuous line, incubation time in sulphate solution (SO_4^{2-}; see Table 1); dashed line, period of exchange from one solution to another. 20 mV ≡ 33·4 kΩ; temperature, 21° C.

the total conductance is relatively small in exhausted fibres. Consequently, only minor potential shifts were observed when external chloride was replaced by sulphate (see Fig. 7) as compared with a transient shift to about -35 mV in normal fibres (cf. Hodgkin & Horowicz, 1959). The relatively small potential shift was also found in a few experiments with isolated single fibres. When instead of replacing Cl^- by SO_4^{2-}, sucrose was isosmotically exchanged for sodium chloride a similarly small decrease in membrane conductance was observed. This confirms the suggestion that the membrane of exhausted fibres is still impermeable to sulphate ions.

In Table 3 the values for potassium and chloride conductance in normal fibres, taken from Hodgkin & Nakajima (1972*a*), are compared with those obtained from exhausted ones. The table shows that the conductance increases for both kind of ions. However, the relation $G_K : G_{Cl}$ changes from 2:3 to 5:1.

The contribution of sodium to the total conductance of the resting membrane has been neglected in the foregoing calculations. This procedure is justified in normal fibres since Hodgkin & Horowicz (1959) could fit their data by applying a modified constant field equation with a permeability ratio $P_{Na}:P_K$ of only 0·01. During the period of repetitive stimulation the internal potassium concentration in poisoned fibres decreased from *ca.* 136 to about 120 m-equiv/kg fibre water and that for sodium increased slightly (R. Fink, unpublished flame photometric experiments) while the membrane potential hyperpolarized or remained constant. The permeability ratio P_{Na}/P_K, consequently, decreased further so that G_{Na} is even less influential under these extreme conditions.

TABLE 3. Membrane conductance in normal and exhausted muscle fibres (μmho/cm^2; temperature, 20–23° C)

A, normal fibres (data from Hodgkin & Nakajima, 1972*a*)

G_m 330	G_K 132	G_{Cl} 198

B, exhausted fibres (present data)

G_{me} 17,200	G_{Ke} 14,300	G_{Cl} 2,900

DISCUSSION

The membrane conductance of a metabolically poisoned muscle fibre increases during a short period of repetitive stimulation to about fifty times its normal value becoming comparable to that during activity. The experiments clearly show that this is mainly due to a hundredfold increase in potassium conductance. In addition the chloride conductance increases but to a lesser extent. Some electrophysiological and electronmicroscopical evidence exists indicating that the ultrastructure of both the surface and tubular membrane remained intact during this procedure:

(*a*) The membranes of exhausted muscle fibres maintained the high Na–K selectivity normally observed under resting conditions. Membrane potentials of -100 mV (external potassium concentration $= 2{\cdot}5$ mM) occasionally observed under these extreme conditions (see Fig. 4 of Grabowski *et al.* 1972) suggest an even higher selectivity.

(*b*) When exhausted muscle fibres were hyperpolarized by passing strong inward current the potassium conductance declined (see Fig. 3) as it is the case in normal fibres with an intact transverse tubular system (Almers, 1972*a*, *b*).

(*c*) The inward rectifier localized mainly in the transverse tubular system (Almers, 1972*a*) could be inhibited in exhausted fibres by Rb and TEA ions in a similar way to that which has been shown in normal fibres (Adrian, 1964; Stanfield, 1970*a*, *b*).

(*d*) Recent investigations with the electron microscope (R. Fink, F. Göbelsmann and H. C. Lüttgau, in preparation) confirm that the tubular system in exhausted fibres remains intact. The ultrastructure of the triad shows no alterations and extracellular markers remain confined to the extracellular space and the T-system.

(*e*) In line with the last observations is the additional finding that the membrane capacity in exhausted fibres remains as high as in normal ones. This shows that the wall of the transverse tubular system is still electrically connected to the surface membrane, as measured with the square pulse method.

In exhausted fibres a potassium conductance of about 14 m-mho/cm^2 (20–23° C) was observed. This value is of the same order of magnitude as the maximum potassium conductance found in normal fibres during depolarization, i.e. 23·2 m-mho/cm^2 (23° C; Stanfield, 1970*a*). It is, therefore, not unreasonable to assume that the increase of potassium conductance in exhausted fibres is due to an activation of existing potassium channels rather than to the formation of new ones.

Recent electrophysiological experiments in skeletal muscle (Adrian *et al.* 1970; Almers, 1972*a*, *b*; Stanfield, 1970*a*, *b*) have revealed the existence of several species of potassium channel with different gating characteristics and different selectivity patterns. The question remains to what extent the increase in potassium conductance observed here can be associated with one of these species of channel. An unambiguous answer to this question can only be given by a voltage-clamp analysis. Some experimental results suggest that the effect might be due to an increase in the conductance of channels which show characteristics of those responsible for the 'slow component'. The reversal potential of this channel species lies close to the resting potential, i.e. it possesses the expected high potassium selectivity. In addition it shows a nearly linear instant current–voltage relation. The 'slow component' appears to be rather insensitive to the blocking action of TEA^+ (Stanfield, 1970*a*, *b*), Rb^+ (Adrian *et al.* 1970) and Zn^{2+} (Stanfield, 1975) so that the considerable potassium conductance which cannot be blocked by these ions might be comparable with activated channels of the slow component.

Finally the reason for the increase in potassium conductance has to be discussed. In muscle fibres with diminished energy reserves the internal free calcium concentration certainly increases. Since it is known that the injection of calcium ions causes a rather specific increase in potassium conductance in different nerve cells (cf. Meech, 1974) it is tempting to associate the effect observed here with an increase in the internal free calcium concentration.

After the injection of calcium ions or the application of caffeine a

relatively small decrease in membrane resistance was found in our experiments. These results may be interpreted as an increase in potassium conductance following an elevation of $[Ca^{2+}]_i$, although until now, there is no unequivocal evidence. In addition, the effect was always smaller than that observed in fibres with diminished energy reserves. It may be that under the latter conditions some unknown reactions are induced (without necessarily excluding that calcium is involved), which activate existing potassium (and possibly chloride) channels. These reactions (e.g. phosphorylations) probably do not affect the selectivity pattern of the channels concerned but interfere with their 'gating' mechanism.

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